Issue of obviousness/non-obviousness

I. The summary of the statements in the Office Action (June 4, 2004) "The instant application appears to take a well known disease and a well known mutation and detect and quantify using well known detection and quantification means. This combination of prior methods does not constitute an unobvious contribution over the art" indicates an unsound basis for rejection. Such reasoning clearly implies that all previous U.S. patents have been wrongly assessed because there is no invention that does not use prior known means or devices previously applied for other purposes to create a new invention. For example, this applicant had previously received the U.S. patent for a method of dissolving preformed beta-amyloid peptide fibrils in vitro by poly-L-lysine (U.S. Patent No. 6, 639, 058). Poly-L-lysine is a well known compound used for a variety of other purposes that was used to dissolve preformed beta-amyloid peptide fibrils found in the brains of people with Alzheimer's disease, a well known disease. A well known device (electron microscopy) was also utilized to observe the dissolution. Poly-L-lysine has also been previously used to bind with protein (histone) and DNA in order to analyze the complexation between DNA and histone in chromatin (Hsueh Jei Li et al., Biochemistry, 1973, 12, 9). Another example concerns the applicant's appropriate combining of well known compounds of gelatin or albumin and trehalose to stabilize the enzymatic activity of acetylcholinesterase (well known enzyme), for which U.S. Patent No. 5,624,831 was granted. Prior to this patent, the same well known compounds were used in a different inventor's U.S. Patent No. 4, 324, 858 and in the publication on "Extraordinary stability of enzymes dried in trehalose" (C. Colaco et al., Biotechnology, 1992, 9, 1007-1013).

II. The statements in the Office Action reflect a misunderstanding of the complexity of the specific disease and obstacles faced by researchers for the development of an adequate

quantitative diagnostic test for SMA disease. Developing this application's quantitative molecular diagnostic method for SMA disease is not a matter of trying different techniques like trying to combine known cooking ingredients to see what concoction could be produced.

Background of SMA disease

Spinal muscular atrophy (SMA) is a neuromuscular disorder characterized by a degeneration of motor neurons from the ventral horn of the spinal cord, leading to symmetrical paralysis of voluntary muscles with muscular atrophy. To date, definitive treatment information about SMA has not been sought consistently. The survival motor neuron (SMN) gene is found to be partially deleted or mutated in over 98% of SMA patients and therefore considered the SMA determining gene.

Duplication of SMN gene and the need for a quantitative diagnostic method

(The following information had been previously provided to the examiner in applicant's letter of 2 Oct. 2003 and fax of 28 May 2004; they also refer to the points mentioned in the Specificiation pages 2 and 12).

The SMN gene duplicates into highly homologous copies – SMNT (telemoric) and SMNC (centromeric). Only homozygous absence of SMNT exons 7 and 8 is responsible for SMA disease. For the diagnosis of SMA, Lefebvre et al. uses the SSCP technique to analyze SMN gene which means working at the DNA level to detect the presence or absence of homozygous deletions of SMNT exons 7 and 8 or only exon 7 (Lefebvre et al., Cell, 1995, **80**, 155-165). However, this qualitative diagnostic method does not allow the identification of heterozygous deletions of SMNT exons 7 and 8 (SMA carriers). This is due to the highly complex problem caused by the duplication of SMN gene. Only a quantitative diagnostic method that permits the precise counting of the number of exons 7 and 8 in SMNT and SMNC

would allow the detection of SMA carriers. Thus, to identify SMA carriers, a few researchers (McAndrew et al., American Journal of Human Genetics, 1997, **60**, 1411-1422; Chen et al., American Journal of Medical Genetics, 1999, **85**, 463-469; Ogino et al., Journal of Molecular Diagnostics, 2001, **3**, 150-157) have tried to use the quantitative PCR technique from the SMN gene, which means working at the DNA level to try to count the number of SMNT and SMNC. However, in order to avoid inaccuracies in the results, this quantitative PCR technique at the DNA level needs a great deal of techniques precautions such as the controls prepared by the same extraction method, the preflashing of the film and monitoring exposure times to ensure the linearity of film response for autoradiography. Furthermore, this method is not suited for diagnostic for purpose to be used in clinical laboratories.

Non-obviousness

As seen above, researchers have continued to focus their study at the DNA level (Specification, page 2). The problem to detect SMA carriers thus remains unresolved.

-What is not obvious is to conceptualize a different approach to develop a quantitative diagnostic method. This is not a matter of using known techniques. Previously, researchers have focused their work at the DNA level, using well known techniques of the field of molecular biology such as the following (as quoted in examiner's Office Action of Final Rejection), and yet have been unable to adequately resolve the problem of detecting SMA carriers: Powell, Gruber, Feuerstein (page 12 Office Action); Bruce, Lefebvre (pp. 13, 14 Office Action).

-What is not obvious is to figure out the right place to focus on, in order to develop an accurate quantitative diagnostic method. The difficulty lies in the highly complex problem of gene duplication inherent in SMA disease. Thus, what is not obvious is the applicant's conceptualization of where to focus on. The applicant's choice is based on the concept

originating from the relationship between the DNA and protein via the intermediary phase of mRNA.

DNA gives mRNA by transcription process, and in turn, mRNA gives protein by translation process:

$$\text{DNA} \xrightarrow[\text{Transcription}]{} \text{mRNA} \xrightarrow[\text{Translation}]{} \text{protein}$$

Therefore, normally, anything that happens at the DNA level will be reflected at the mRNA level and at the protein level. If there is a problem such as mutation or deletion at the DNA level, this problem will also be found at the mRNA level, and the protein will be non-functional thus leading to disease. However, in certain cases what happens at the DNA level cannot be found at the mRNA level due to mRNA editing (S. Maas, A. Rich, Bioessays, 2000, **22**, 790-802) or due to stop codon suppression (D.N. Robinson, L. Cooley, Development, 1997, **124**, 1405-1417; S.L. Alam, N.M. Wills, J.A. Ingram, et al. J. Mol. Biol., 1999, **288**, 837-852); either case leads to a functional protein. This is the reason why it is important to consider working at the mRNA level.

Regarding SMA disease, 98% of SMA patients carry homozygous deletions of exons 7 and 8 or only exon 7 of SMNT, either because of conversion of sequences in SMNT to those in SMNC, or as a result of SMNT deletions (Lefebvre et al., Cell, 1995, **80**, 155-165).

This applicant thus decided to pursue to place the work focus at the mRNA level based on the observation that the SMNT transcripts (SMNT-mRNA) were absent and that the SMNC transcripts (SMNC-mRNA) were solely present in SMA patients lacking the SMN gene on both mutant chromosomes, while control individuals expressed both RNA transcripts, SMNT-mRNA and SMNC-mRNA (Lefebvre et al., Cell, 1995, **80**, 155-165). Furthermore, the SMNC

transcripts, not the SMNT transcripts, may undergo alternative splicing of exon 7 to produce transcripts lacking this exon.

Non-obvious next steps

Once this applicant decided that the work should be done at the mRNA level, what is not obvious is to identify the approach to develop a quantitative diagnostic method.

Jong et al. (pp. 10, 11, 15 Examiner's Office Action; applicant's letter of 2 Oct. 2003; applicant's fax 24 May 2004), in a semi-quantitative research method using Image Analysis System, analyzed the mRNA transcripts of the SMN gene in the peripheral blood mononuclear cells of normals, carriers and SMA patients:

Total RNA

↓
RT process using **RANDOM** primer PolydT which results in cDNA
NON-SPECIFIC

↓
PCR process using different specific primers and the biotin-labeled probes to locate the SMN fragments
----- -- Exon 7-- -------- ----------
↓

After locating which ones are SMN fragments (by Southern blot analysis and by using antibodies anti-biotin), performing the sequencing to detect the presence of exon 7 in the above mentioned different PCR fragments.

Then, they examined lighter or darker shades of bands 355 bp (presence of exon 7) and 301 bp (absence of exon 7), and 419 bp (presence of exon 7) and 365 bp (absence of exon 7) of PCR fragments. Such examination of the lighter or darker shades of the PCR fragments in 3% agarose gels does not give accurate results; examination of lighter or darker shades of the bands that are so close to each other (355 bp and 301 bp; 419 bp and 365 bp) does not give precise results (see figures 2.B and 2.C page 150, Jong et al., Journal of Neurological Sciences, 2000,